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14. ABSTRACT

Breast cancer is one of the leading causes of death for women in the United States and estrogen exposure has been hypothesized to be involved in the development of this cancer. Our lab is studying the ACI rat, an estrogen-induced breast cancer animal model to begin to elucidate estrogen's role in breast cancer. The ACI rat develops mammary cancer after prolonged exposure to 17β-estradiol, while the BN and genetically related COP rats do no. We have identified several polymorphisms in the promoter region of *p16*^{cdkn2a} between the ACI rat and either the BN or COP rats. These polymorphisms lead to changes in expression and methylation of *p16*^{cdkn2a}.

15. SUBJECT TERMS
Hypermethylation, P16INK4A, CDKN2A, tumor suppressor, estrogen, mammary cancer, epigenetics

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The ACI rat is uniquely susceptible to estrogen (E2) induced mammary carcinogenesis while the BN and genetically related COP rats are not as susceptible. Genetic studies utilizing these strains have allowed us to identify a region on rat chromosome 5, containing *p16*^{cdkn2a}, that confers susceptibility to E2 induced mammary cancer. *P16*^{cdkn2a} has been identified as a tumor suppressor gene that functions as an inhibitor of CDK4 and CDK6 and controls the G_1/S transition of the cell cycle (1). Loss of *p16*^{cdkn2a} expression has been shown to occur in human breast cancer and is most commonly reported to be due to hypermethylation (1). We have previously demonstrated that the expression of p16^{cdkn2a} is dramatically down regulated at the protein level at an early stage of E2-induced mammary carcinogenesis, whether or not this occurs at the mRNA level and the mechanism behind this down regulation has yet to be identified. Our hypothesis is that the mRNA levels of *p16*^{cdkn2a} decrease upon exposure to E2 in the ACI strain in the focal regions of atypical hyperplasia, carcinoma *in situ*, and invasive carcinoma. In addition, we hypothesize that the mRNA levels in the BN and COP strains will not decrease upon the same time of exposure to E2. Furthermore, we hypothesize that this loss of expression is due to methylation of the promoter and exon1 of *p16*^{cdkn2a} in an estrogen dependent manner. To begin to address this hypothesis, we proposed to determine the effect of E2 on the mRNA expression of *p16*^{cdkn2a} in mammary tissue of the ACI, COP and BN strains, to determine the methylation pattern of the promoter and exon 1 of *p16*^{cdkn2a} in the ACI, COP and BN strains of rats in normal, lobular hyperplasia, atypical hyperplasia, carcinoma *in situ*, and invasive carcinoma.

Differences in Methylation of DNA from nontumor and tumor tissue from E2 treated ACI rats. Experiments from the previous reporting period indicated differences in the methylation status from nontumor and tumor tissues from E2 treated ACI rats. These experiments were repeated in F1 (ACI x BN) in order to generate some preliminary data on differences in methylation that may occur in the ACI and BN rats due to polymorphisms. As shown in figure 1, the ACI allele is significantly more methylated than the BN allele in the tumor tissues in the F1 population, while there was no significant difference in the nontumor tissues.

Polymorphisms lead to changes in *p16*^{cdkn2a} expression in the ACI, BN, and COP rats in mammary, pituitary, thymus, lung and spleen tissues. Using ACI, BN, and COP rats as well as congenic rats in which the *p16*^{cdkn2a} region from the BN or COP rat was replaced by the same region in the ACI (labeled Emca1). These rats were used to determine if the polymorphisms lead to changes in expression or if another epistatic factor is playing a role. In all cases there was no difference in the Emca1 strains and the background strain in which it was generated. This indicates that the polymorphisms between ACI, BN, and COP lead to changes in *p16*^{cdkn2a} expression.

Key Research Accomplishments

- Determined methylation status of F1 E2 treated rats
- Determined expression of *p16*^{cdkn2a} in ACI, BN, COP and Emca1 rats

Reportable Outcomes

None

Conclusions

The polymorphisms we identified previously lead to changes in methylation and expression of *p16*^{cdkn2a}.

Due to difficulties with my current mentor, I have decided to not continue in the laboratory of James Shull and will be pursuing other opportunities.

References

1. Rocco, JW, D. Sidransky. 2001. P16 (MTS-1/CDKN2/INK4a) in Cancer Progression. **Exp. Cell Res.** 264: 42-55

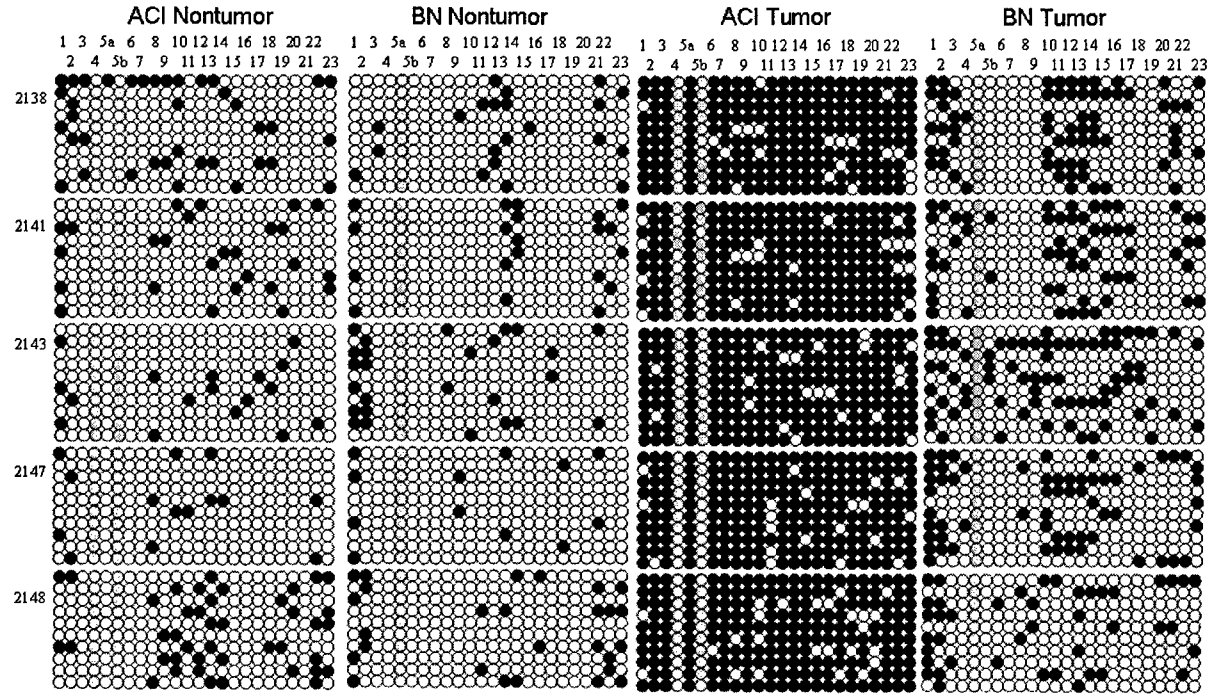

Figure 1. Methylation status of F1 tumor and nontumor tissue. Rat number is on the far right and CpG sites are labeled on the top of each set of tissues. Closed circles are methylated, open circles are not methylated and circles filled with grey are CpG sites that are not found in the ACI or BN rat due to polymorphisms.

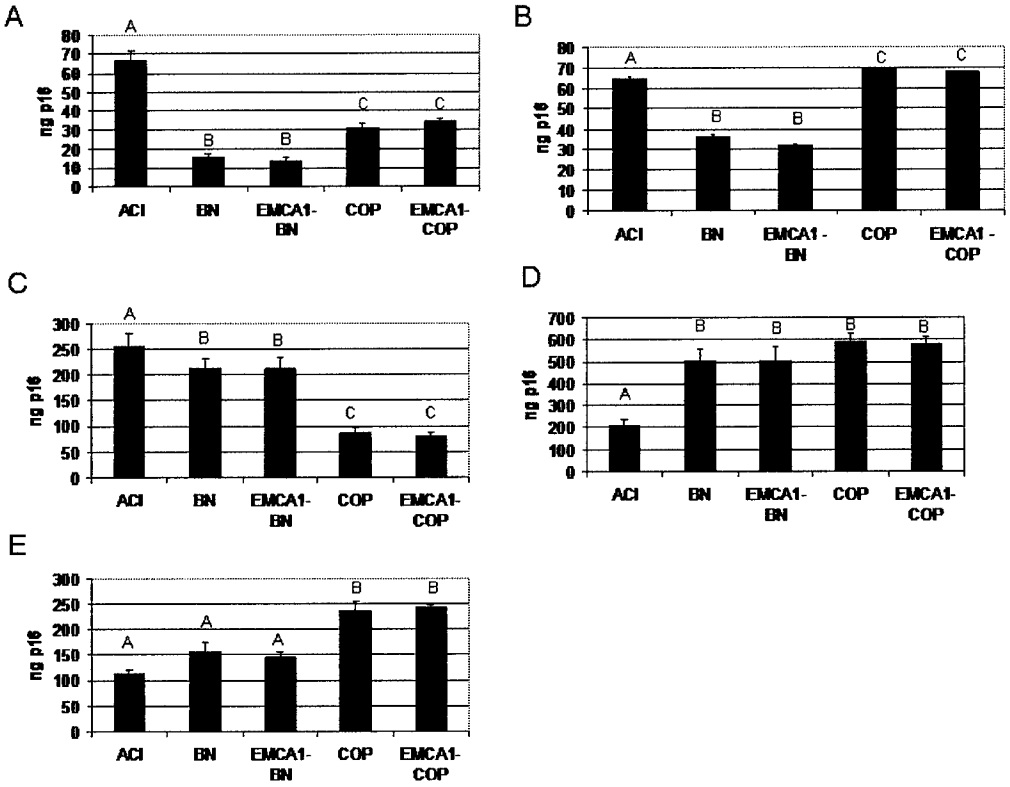

Figure 2. Expression levels of *p16*^{cdkn2a} in mammary (A), spleen (B), lung (C), thymus (D) and pituitary (E). Bars with the same letters are not significantly different in expression.